THE EFFECT OF X-RAYS, IRRADIATED SEA WATER, AND OXIDIZING AGENTS ON THE RATE OF ATTACHMENT OF BUGULA LARVAE[1]

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Few observations concerning the effects of ionizing radiations have been made on bryozoan tissues. Oka (1954) x-rayed various regions of the fresh-water bryozoan, *Lophopodella carteri,* and noted that the more active embryonic tissues had greater radiosensitivity than other parts. But no observations known to the writer have been reported concerning the effects of x-radiation on the attachment and metamorphosis of bryozoan larvae.

It is well known that some of the biological effects of ionizing radiations have been attributed to the production of H_2O_2 or organic peroxides when aqueous solutions are x-rayed (Evans, 1947; Barron *et al.,* 1949; Kimball and Gaither, 1952). Barron and his co-workers found that both irradiated sea water and hydrogen peroxide inhibited oxygen uptake in sea urchin sperm; but the latter had two opposite effects, increasing respiration at great dilution and inhibiting oxygen uptake in higher concentration. Furthermore, Blum (1941, p. 96) has emphasized the probability that the effects of certain photodynamic dyes may be mediated through the production of H_2O_2. Since some of the basic dyes had been found to be potent inductors of attachment and metamorphosis of *Bugula* larvae when sea water solutions of these dyes were exposed to light (Lynch, 1955a), it was of interest to determine whether x-rays and hydrogen peroxide would have any effect on setting. If ionizing radiations were found to affect the rate of attachment of the larvae, the problem of determining whether the action of these rays was a direct or an indirect one would naturally arise. After x-raying the larvae proved to have a positive effect on the rate of setting, it was decided to employ sea water seeded immediately after being x-rayed with non-irradiated larvae. The results of these experiments led to an investigation of the possible effects of adding H_2O_2 to sea water and finally to observations on the action of two other oxidizing agents, sodium 2,6-dichlorobenzenoneindophenol and 2,3,5-triphenyltetrazolium chloride, on the attachment and metamorphosis of *Bugula* larvae.

Materials and Methods

Each experiment on the effects of radiation involved three dishes: the first contained larvae x-rayed in filtered sea water; the second contained sea water that was seeded immediately after being x-rayed with non-irradiated larvae, and the

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third had control larvae in their natural medium. For these experiments plastic containers 7 cm. in diameter and 1.6 cm. high were employed. Each dish contained 30 ml. of sea water and was covered with a plastic top; all three containers were kept close together on a table and were wrapped with paper towels until the time of counting. Light was excluded to delay the decomposition of any photolabile by-products, especially peroxides, that might be formed by irradiation.

The x-ray data are as follows: the machine operates on 182 kv. pk. and 25 ma. with an equivalent filtration of 0.2 mm. of copper. During the summer of 1956, when larvae of *B. turrita* were employed, the output of the tubes (position A) was 4724 r per minute and the organisms were irradiated for three minutes and twenty seconds to give a total of 15,733 r. During the previous summer the x-ray dosage for larvae of *B. flabellata* was 18,333 r and the organisms were also irradiated for three minutes and twenty seconds at 5500 r per minute. The tubes were water-cooled and an electric fan was directed upon them. Since the temperature was found to rise only a fraction of a degree, the irradiated material was not cooled by an ice chamber.

Sea water solutions of the three oxidizing agents were made in the following concentrations found to be most effective: 1:14,000 parts by volume of 30% H_2O_2 (7×10^{-4} *M*), 1×10^{-5} *M* 2,3,5-triphenyltetrazolium chloride (TTC) and 0.01 mg. of sodium 2,6-dichlorobenzenoneindophenol (SDBI) per liter (3.4×10^{-8} *M*). The pH was that of natural sea water. Stender dishes 6 cm. in diameter containing 30 ml. of solution were seeded with larvae and covered with glass lids. The controls were placed in the same amount of sea water in similar containers and kept as near as possible to the experimental dishes. In the experiments with H_2O_2 both experimental and control dishes were shielded from light by wrapping them in paper towels. The others were exposed to diffuse daylight coming from a window about three feet from the region where the Stender dishes were placed.

Results

I. X-rays and irradiated sea water

Table I shows that x-raying larvae of *B. flabellata* induced more rapid setting than that which occurred in the controls, the *t* ratio for the difference of the two groups being 5.12 ($P = .005$). For these experiments the number of attached organisms was counted thirty minutes after irradiation with 18,333 r. The three experiments in which larvae were placed in sea water immediately after it had been irradiated suggest that the accelerated rate of attachment is an indirect effect presumably caused by the action of ionizing radiations on sea water.

Table II gives more convincing evidence of an indirect effect of ionizing radiations. For these experiments larvae of *B. turrita* were irradiated with 15,733 r and the number of attached organisms was counted at the end of eight hours. The time of irradiation was the same for both groups of larvae, but it was found that the output of the x-ray tubes had dropped during the course of a year when the machine was calibrated towards the end of the period of experimentation. Although the rate of attachment of the larvae of both *B. flabellata* and *B. turrita* was accelerated either by x-raying the larvae or by seeding them in irradiated sea water, the time at which the effects could be detected differed considerably. Larvae of

B. turrita showed no notable acceleration of the rate of attachment by thirty minutes after either being x-rayed or being placed in irradiated sea water, but by eight hours there were always more attached organisms in the experimental dishes than in the controls. The *t* ratio for counts made at this time indicates a significant difference in the mean number of attached organisms in the experimental and control dishes, being 6.64 (P = .001) for irradiated larvae and 4.09 (P = .005) for organisms seeded in irradiated sea water. It does not appear that the difference in the rate of setting of *B. flabellata* and *B. turrita* can be ascribed to the lower x-ray dosage of the latter, for 20,000 r did not produce effects appreciably different from those which followed irradiation with 15,733 r. After studying the effects of various agents on both types of larvae, one gains the general impression that it is both

TABLE I

The effects of x-rays (18,333 r) and of irradiated sea water on the rate of attachment of larvae of B. flabellata. The larvae were irradiated thirty minutes after the adult colonies had been exposed to light and the irradiated sea water was seeded with larvae at the same time. The number of attached organisms in the three groups (x-rayed larvae, larvae in irradiated sea water and those in natural sea water) was counted thirty minutes later

X-rayed larvae				Larvae in irradiated sea water			Control larvae		
No. of exp.	No. of larvae	No. attached	Per cent attached	No. of larvae	No. attached	Per cent attached	No. of larvae	No. attached	Per cent attached
1	104	87	84	87	85	98	100	50	50
2	214	207	97	66	63	95	63	62	98
3	46	44	96	191	190	99	145	48	33
4	150	143	95	—	—	—	349	183	52
5	248	242	98	—	—	—	247	158	64
6	340	330	97	—	—	—	257	130	50
7	170	158	93	—	—	—	167	55	33
8	151	141	93	—	—	—	94	20	21
	Average per cent		94 ± 4						50 ± 24

The *t* ratio for the significance of the difference of the means of the x-rayed and control larvae = 5.12; P = <.005. The *t* ratio was computed from percentages carried out to one decimal point (not the rounded percentages shown in columns 4 and 10).

more difficult to induce metamorphosis and easier to inhibit fixation in larvae of *B. turrita* than in those of *B. flabellata*. These differences may be correlated with the longer natatory period of *B. turrita* in natural sea water. It is difficult to determine, however, whether these differences are actually specific or whether they can be attributed to altered environmental conditions that prevailed during the two summers when each type was used almost exclusively.

Although either x-raying larvae of *B. turrita* or seeding them in irradiated sea water induced more rapid attachment of the organisms, subsequent development was seriously impeded. Frequently larvae that had been x-rayed failed to develop after attachment. In other cases undifferentiated growth occurred at a much retarded rate. Instead of forming normal zoids, the larvae merely developed elongated masses of clear, gelatinous, stolon-like material without any internal organization.

Usually these growths formed at opposite sides of the body of the larva. One growth evidently corresponded to the stolon for attachment of the organism and the other developed in the region where the body of a normal zoid usually forms. Both growths were abnormally long and slender. The material corresponding to the stolon of a normal zoid rarely differentiated into the four knob-like projections, symmetrically placed and each branching dichotomously, which are characteristic of stolons of *B. turrita.* (Stolons of *B. flabellata* have three rather than four parts.) The material which grew out from the region where the zooecium normally forms did not differentiate into a gut and tentacles.

TABLE II

The effect of x-rays (15,733 r) and of irradiated sea water on the rate of attachment of larvae of B. turrita. The larvae were irradiated thirty minutes after the adult colonies had been exposed to light and the irradiated sea water was seeded with larvae at the same time. The number of attached organisms in the three groups (x-rayed larvae, larvae in irradiated sea water and those in natural sea water) was counted eight hours later

Temp. = 24–26° C.

X-rayed larvae				Larvae in irradiated sea water			Control larvae		
No. of exp.	No. of larvae	No. attached	Per cent attached	No. of larvae	No. attached	Per cent attached	No. of larvae	No. attached	Per cent attached
1	102	100	99	75	65	87	36	12	33
2	60	56	93	194	192	99	48	38	79
3	26	23	88	140	130	93	31	17	55
4	41	35	85	102	101	99	46	28	61
5	15	14	93	56	48	86	26	16	62
6	45	36	80	131	76	58	43	15	35
7	32	28	88	180	154	85	28	20	71
8	57	43	75	96	65	88	47	19	40
9	49	43	88	78	69	88	50	20	40
10	24	21	88	20	10	50	22	15	68
11	45	40	89	23	14	61	55	22	40
12	50	45	90	24	19	79	41	29	71
	Average per cent		88 ± 6			81 ± 16			54 ± 16

The *t* ratio for the significance of the difference of the means of the x-rayed and control larvae = 6.64; P = <.001. The *t* ratio for larvae in irradiated sea water vs. the controls = 4.09; P = .005. The *t* ratios were computed by using the rounded percentages in columns 4, 7, and 10.

Larvae that attached in irradiated sea water developed similar undifferentiated growths. These zoids, however, elongated more than those formed from larvae that had been x-rayed. In fact, growth sometimes exceeded that of the controls, but the transparent gelatinous material, often peculiarly twisted, lacked internal organization. Larvae that attached to the surface film sometimes developed normal stolons. Those that attached to the bottom frequently formed long slender stolons, about three times normal length, with a spherical mass in the region where they were attached to the substrate; and some were attached by two stolons. A few developed a bud in the zooecial wall for a second zoid. But even when the original irradiated sea water had been replaced several times by fresh sea water, the growth

corresponding to the zooecium failed to differentiate a gut and tentacles. Only a single larva formed a well-developed zoid with everted tentacles; and this differentiation occurred only after six days, whereas internal organization can readily be detected in a normal zoid by the end of forty-eight hours. Thus, a notable feature of larvae that were either x-rayed or placed in irradiated sea water was growth without differentiation; and the development of x-rayed larvae was more drastically impeded than that of organisms seeded in irradiated sea water.

These observations seem to be reasonably consistent. Nevertheless, it would be premature to ascribe the failure of the zoids to differentiate in a normal manner solely to ionizing radiations. X-raying the larvae not only markedly reduces their motility but also causes them to settle on the bottom of the container, and larvae which attach geopositively usually do not develop as well as those which affix themselves to the surface film. Factors affecting larval differentiation are at present largely unknown. And judgments concerning degrees of growth are more subjective than those based on numerical data. In an almost unexplored field of larval differentiation, experimental designs that appear to have only one variable may be deceptive unless similar replications can be obtained during two different summers and with more than one species. Since the chief purpose of the experiments was to determine the effects of x-rays and irradiated sea water on the attachment of the larvae, it would be inadvisable to draw definitive conclusions concerning the influence of these agents on development until further observations have been made.

A few experiments were performed to determine what role the surface of the plastic containers might play in attachment of the larvae. Both x-rayed larvae and organisms irradiated in sea water were emptied from the plastic containers into Stender dishes and the latter were also used for the controls. Although the larvae attached somewhat less readily in the Stender dishes, there was more rapid fixation of both x-rayed larvae and those in irradiated sea water than in the controls. It is not unlikely that some of the great variability in the time of attachment of the controls, always a puzzling situation that occurs yearly in almost every experiment, can be attributed to differences in roughness of the various Stender dishes used. The temperature of the water in which the adult colonies are kept also seems to cause variability in the time of setting (*cf.* Lynch, 1955b).

II. Oxidizing agents

One of the problems encountered in determining the possible effects of oxidizing agents on the rate of fixation of the larvae was that of getting solutions dilute enough to prevent cytolysis. Both strong and weak solutions greatly reduced the motility of the larvae. But in solutions that were too strong the larvae merely became immobilized on the bottoms of the containers without attaching themselves; and these organisms eventually cytolyzed. With solutions that were weaker the time of counting was an important factor. If counts were made too soon, no observable differences in control and experimental larvae could be detected. If counts were made too late, when the controls had metamorphosed in large numbers, again no differences could be observed. After a wide variety of concentrations had been tested, the right dilution for inducing attachment was eventually found.

Hydrogen peroxide. Table III shows that by twenty-one hours the number of attached larvae (*Bugula turrita*) in sea water containing 1:14,000 parts of 30% H_2O_2 was significantly greater than that of the controls ($P = .001$). Since there were no notable differences in the number of attached organisms in the control and experimental dishes by twelve hours, H_2O_2 had a delayed action in inducing fixation, somewhat similar to that observed after one-minute exposures of bryozoan larvae to urea (Lynch, 1957). The cause of this delayed action is unknown.

Zoids formed from larvae exposed to sea water containing H_2O_2 resembled those in irradiated sea water. Elongation sometimes exceeded that of the controls, but the zoids were usually misshapen. The larvae generally attached in greater numbers to the surface film than to the bottoms of the containers and the majority

TABLE III

The effects of 30% H_2O_2 (1:14,000 parts by volume = 0.0072 volume per cent) in sea water on the rate of attachment of larvae of B. turrita. The experimental and control solutions were seeded with larvae thirty minutes after the adult colonies had been exposed to light (except no. 1, which was seeded at fifty minutes). The number of attached organisms was counted twenty-one hours later. The pH was that of sea water

No. of exp.	Experimental larvae			Control larvae		
	No. of larvae	No. attached	Per cent attached	No. of larvae	No. attached	Per cent attached
1	28	28	100	23	12	52
2	62	54	87	32	15	47
3	35	34	97	72	38	53
4	16	16	100	13	6	46
5	31	31	100	17	11	65
6	20	20	100	20	18	90
7	37	36	97	43	20	46
8	35	35	100	44	22	50
	Average per cent		98 ± 2			56 ± 15

The *t* ratio for the significance of the difference of the means of experimental and control larvae (computed by using fractional percentages) = 7.07; P = <.001

of these formed normal tetrapod stolons with dichotomous branches at their ends, but the zooecial region failed to differentiate internal organs. Geopositive larvae produced much gelatinous, stolon-like material without differentiation. None of the organisms observed during a period of a week developed tentacles, although these normally form by about forty-eight hours. Larvae of *B. flabellata,* observed during the previous summer, were less adversely affected than those of *B. turrita.* Although H_2O_2 considerably reduced the motility of the larvae, it apparently was not excessively injurious to the cilia; otherwise the organisms would have dropped to the bottoms of the containers and remained there.

The effects of TTC. Table IV shows that sea water solutions of TTC in concentrations of 1×10^{-5} *M* (pH = 7.9–8.0) also induced more rapid fixation of the experimental larvae (*B. turrita*) than that which occurred in the controls. In solutions of 5×10^{-5} *M* the larvae attached more readily than in the weaker medium,

TABLE IV

The effect of 0.00001 M 2,3,5-triphenyltetrazolium chloride in sea water (pH = 7.9–8.0) on the rate of attachment of larvae of B. turrita. Control and experimental dishes were seeded with larvae thirty minutes after the adult colonies had been exposed to light and the number of attached organisms in each group was counted at the end of eight hours

No. of exp.	Experimental larvae			Control larvae		
	No. of larvae	No. attached	Per cent attached	No. of larvae	No. attached	Per cent attached
1	61	49	80	33	17	52
2	36	24	67	35	11	31
3	41	19	46	27	8	30
4	55	35	64	23	8	35
5	50	42	84	37	17	46
6	30	26	87	56	38	68
7	44	41	93	58	34	59
	Average per cent		74 ± 15			46 ± 16

The *t* ratio for the significance of the difference of the means of the control and experimental larvae (computed by using fractional percentages) = 3.47; P = .015

but subsequent development was considerably retarded. If the Stender dishes were flooded with fresh sea water after the larvae had attached, growth took place at a markedly reduced rate after exposure to either concentration of TTC. Some of these organisms formed tentacles. Larvae left in the weaker of the two TTC

TABLE V

The effect of sodium 2, 6-dichlorobenzenoneindophenol in concentrations of 0.01 mg./liter of sea water (3.4×10^{-8} M) on the rate of attachment of larvae of B. turrita. The experimental and control Stender dishes were seeded with larvae thirty minutes after the adult colonies had been exposed to light and the number of attached organisms was counted seven hours later. Temp. = 24–26° C. The pH of the experimental solution was that of sea water

No. of exp.	Experimental larvae			Control larvae		
	No. of larvae	No. attached	Per cent attached	No. of larvae	No. attached	Per cent attached
1	56	50	89	42	24	57
2	81	59	73	53	16	30
3	64	31	48	35	11	31
4	49	42	86	115	28	24
5	82	46	56	97	55	57
6	128	92	72	96	47	49
7	82	53	65	197	132	67
8	205	127	61	61	36	59
9	58	44	76	64	34	53
10	18	17	94	44	27	61
	Average per cent		72 ± 15			49 ± 17

The *t* ratio for the significance of the difference of the means of the experimental and control larvae (obtained by using rounded percentages in columns 4 and 7) = 3.08; P = .015.

solutions elongated slightly by twenty-four hours and these zoids resembled those in sea water containing H_2O_2 insofar as there was an abnormal amount of gelatinous material without differentiation; organisms left in the stronger solution did not develop. Larvae that attached in solutions of 5×10^{-5} *M* TTC became slightly pink, indicating a reduction of the solution; those in the weaker solution became faintly pink. Although TTC solutions exposed to air turned somewhat pink by eight hours, either with or without organisms in them, the larvae were always more deeply colored than the solutions.

The effects of SDIB. This oxidizing agent, while inducing fixation of the larvae at a rate significantly higher than that of the controls ($P = .015$), as shown in Table V, was less injurious to the larvae than any of the other agents discussed in this paper. Larvae that were left in solutions of 0.01 mg./liter of sea water ($pH = 7.9$) generally developed normally but at a rate somewhat slower than the controls. A preponderance of settings occurred at the surface film, and these developed better than those on the bottom. Larvae that attached to the bottoms of the Stender dishes formed zoids without any differentiation by forty-eight hours. But development was variable in these solutions, sometimes equalling that of the controls and sometimes being inferior.

III. Reducing agents

A very limited number of preliminary experiments was carried out with two reducing agents, sodium bisulfite and sodium thiosulfate, to determine whether their action on attachment would be opposite to that of oxidants. But neither reducing agent prevented attachment. Sea water solutions of sodium thiosulfate as strong as 10 mg./liter and concentrations of sodium bisulfite of 0.0001 *M*, 0.001 *M* and 0.005 *M* did not prevent attachment and metamorphosis. Nor did these solutions appear to have any inhibitory effects on the larvae. Although it seems unlikely that further experimentation with these solutions will show that they inhibit attachment, a greater variety of concentrations should be tested before a definitive conclusion can be reached concerning their action.

Discussion

Since the action of x-rays in inducing fixation of *Bugula* larvae can be simulated by irradiated sea water, the effect on attachment appears to be an indirect one. Other instances of a similar indirect effect of ionizing radiations in living material have been reported in the literature. Barron and his co-workers, for instance, believed that the inhibiting effect of x-rayed sea water on the respiration of sea urchin sperm was attributable to stable organic peroxides formed during irradiation of the medium (Barron *et al.*, 1949). Similarly, Wichterman and Figge (1954) found that when paramecia were x-rayed the lethality of the radiations was correlated with the extent of exposure to air of the culture medium. These investigators concluded that the lethal factor was probably H_2O_2 or some other oxidation product formed in the moist air surrounding the culture fluid; their paper contains a brief review of the literature. The apparently indirect action of x-rays in inducing fixation of bryozoan larvae may offer a possible explanation of the seemingly strange observation of Bertholf and Mast (1944) that extracts of

muscle tissue from rabbits killed with x-rays had an accelerating effect on ascidian metamorphosis.

The action of hydrogen peroxide and the other oxidizing agents discussed in this paper poses an interesting question concerning the effects of four other agents in inducing metamorphosis: copper, basic dyes, iodine and quinone (Lynch, 1949, 1952, 1956, 1957). These substances are also inhibitors of the dehydrogenases, and the action of some of them can be reversed by cysteine (references for copper and iodine are given by Needham, 1950, p. 425; for quinone see White *et al.*, 1954; and for basic dyes *cf.* Quastel and Wheatley, 1931). The difference in effect on attachment produced by cysteine on the one hand (Lynch, 1957) and by iodine, copper and the basic dyes on the other, might be an indication that the latter inactivate an enzyme system, such as succinic dehydrogenase, and that this inactivation abruptly ends larval life allowing the adult action system to gain control. Such a hypothesis has been proposed by Glaser and Anslow (1949) as a possible explanation of the action of copper in inducing metamorphosis in ascidian tadpoles; these investigators have suggested that copper may operate alternately as an electron donor or acceptor in the prosthetic group of some oxidizing enzyme, which they visualize as being a porphyrin-like ring compound. The data on bryozoan metamorphosis do not preclude the possibility that oxidizing enzymes may be involved, but it seems unlikely that succinic dehydrogenase plays an important role in the fixation of *Bugula* larvae, for this enzyme is inhibited by urethane (White *et al.*, 1954). If the effect were primarily on this enzyme system, one would expect urethane to act like quinone, copper, iodine and the basic dyes; but urethane inhibits fixation, whereas the other four agents induce attachment (Lynch, 1957).

The effect of these agents on succinic dehydrogenase in other organisms, however, may give a clue concerning their action on the colloidal state of protoplasm. It has long been suspected that oxidizing and reducing agents play an important part in blood clotting (Matthews, 1936). And it has been suggested that the conversion of fibrinogen into fibrin involves an oxidation process. Chargaff and Bendich (1943) believe that this oxidation involves aminoacyl groups of proteins; Baumberger (1941), on the other hand, considers that the oxidation of sulphydryl groups is of prime importance in the clotting mechanism. And the significance of certain reducing agents which inhibit the conversion of prothrombin into thrombin has been emphasized by Carter and Warner (1954). Recently Mazia and Dan (1952) have shown that the spindle fibers of cells undergoing mitosis can be isolated by creating artificial disulfide bonds when these cells are treated with H_2O_2 (or iodine) before the rest of the cell content is solubilized by a detergent. These investigators believe that H_2O_2 removes hydrogen from the sulphydryl groups of proteins and converts these substances into less soluble material by joining them together by —S—S— bonds. Thus there appears to be a polymerization of smaller molecules through these disulfide bridges. Calcutt (1951), likewise, thinks that certain photodynamic dyes affect the exposed —SH groups of the protein molecule.

The diversity of factors capable of inducing attachment of bryzoan larvae seems to indicate that these agents have a direct effect on the fluid of attachment. In many of the sessile organisms the cementing substance appears to be a mucoprotein (*cf.* Pyefinch and Downing, 1949), and Knight-Jones (1953) has found evidence that barnacles attach by means of a substance which he considered to be a quinone-

tanned protein, a compound similar to the material in the hardened cuticle of an insect. The action of x-rays in inducing fixation of *Bugula* larvae would not be out of harmony with the working hypothesis that attachment, when artificially induced, is brought about by agents which cause coagulation. Such a coagulating effect of x-rays has been reported for such varied types of protoplasm as sea urchin eggs (Rieser, 1955) and paramecia (Wichterman and Figge, 1954). On the other hand, irradiation of fibrinogen prolongs the clotting time (Rieser, 1956). The possibilities just discussed may form a link which would connect the effect of photodynamic dyes with that of x-rays, since both agents may release H_2O_2 or organic peroxides (Blum, 1941, p. 96; Barron *et al.*, 1949). It would be reasonable to suspect that the action of both iodine and quinone would be similar to that of H_2O_2.

The excellent development of zoids formed from larvae whose metamorphosis had been induced by treatment with sea water containing neutral red in parts of 1:100,000 (Lynch, 1952) and the poor development of zoids in solutions of H_2O_2 may be attributed, perhaps, either to unrecognized extrinsic factors affecting the latter or to the higher concentration of H_2O_2 (1:14,000 parts). Experiments with concentrations of neutral red that were ten times stronger than those used for the observations previously reported showed that larvae in these media also failed to develop after attachment.

Summary

1. X-raying larvae of either *B. flabellata* (18,333 r) or *B. turrita* (15,733 r) within thirty minutes after the organisms began to emerge from the parental colonies induced more rapid setting than that which occurred in the controls ($P = .005$ and .001, respectively). Irradiated sea water had a similar, but slightly less pronounced, effect ($P = .005$). In these experiments the subsequent development of larvae of *B. turrita* into zoids was drastically impeded. Slow growth, usually without differentiation, was observed.

2. Sea water solutions of H_2O_2 (7×10^{-4} *M*), of 2,3,5-triphenyltetrazolium chloride (1×10^{-5} *M*) and of sodium 2,6-dichlorobenzenoneindophenol (3.4×10^{-8} *M*) at a pH of 7.8–8.0 also induced more rapid setting of the experimental larvae ($P = .001$, .015 and .015, respectively). The subsequent development of larvae exposed to H_2O_2 and to 2,3,5-triphenyltetrazolium chloride resembled that of organisms that were either x-rayed or placed in irradiated sea water. Sodium 2,6-dichlorobenzenoneindophenol was less injurious to the larvae than the other agents used. An explanation of the possible role of these agents in inducing an accelerated rate of setting is presented.

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